CYTOLOGICAL STUDIES OF NATURAL INTERGENERIC HYBRIDS AND THEIR PARENTAL SPECIES IN THE MOSS GENERA, *ASTOMUM* AND *WEISSIA*[1]

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The relationships of a complex of species within or near the moss genus, *Weissia* Hedw., have puzzled bryologists for more than a hundred years. The main problem is the uncertain status of two segregate genera, *Hymenostomum* R. Br. and *Astomum* Hampe. Recurring reports of intergeneric hybrids between species of all three genera have tended to weaken the case for maintaining separate genera, yet the segregate genera are based on characters which usually are considered strong. An opportunity to study meiosis in two natural intergeneric hybrids, *Astomum ludovicianum* Sull. × *Weissia controversa* Hedw. and *A. muhlenbergianum* (Sw.) Grout × *W. controversa*, initially prompted this study.

Taxonomic relationships within the *Weissia* complex and arguments for and against maintaining one or more of the segregate genera have been discussed by Lindberg (1879), Andrews (1920, 1922, 1924, 1933), Hilpert (1933), Grout (1938), Jensen (1939), Chen (1941), Steere, Anderson and Bryan (1954), Podpěra (1954), Nyholm (1956), Demaret and Castagne (1964), Reese and Lemmon (1965), and Williams (1966). The distinguishing characters of the three genera, as presently understood, are summarized in Table 1.

Astomum includes species with immersed capsules in which an operculum is lacking or very poorly differentiated and non-functional. Capsules are cleistocarpous, breaking open irregularly at maturity. Peristome is lacking, and there is no membranous covering or "hymenium" at the mouth of the capsule.

Hymenostomum embraces species with exserted capsules, a functional operculum, and a thin membrane which covers all or a portion of the mouth of the capsule. Annulus and peristome are lacking.

Weissia contains species with exserted capsules, a differentiated operculum, annulus, and peristome, although the latter is sometimes much reduced in size, or rarely absent.

Distinguishing gametophytic characters are lacking, if all species are considered. Gametophytes of the two North American species of *Astomum*, how-

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TABLE 1. Distribution of sporophytic characters in the three genera of the *Weissia* complex.

	ASTOMUM	*HYMENOSTOMUM*	*WEISSIA*
Seta	short, capsule immersed	short to long, capsule emergent to exserted	short, capsule exserted
Capsule shape	subglobose to oblong-cylindric	rounded-ovate to long-cylindric	rounded-ovate to long-cylindric
Operculum	absent to slightly differentiated	present	present
Annulus	absent	absent	present
Oral membrane	absent	present	absent
Peristome	absent	absent	present

ever, are distinctive and are generically recognizable, although *A. ludovicianum* and *A. muhlenbergianum* can scarcely be told apart without sporophytes. Compared with the North American species of *Weissia* and *Hymenostomum*, the leaves of *A. ludovicianum* and *A. muhlenbergianum* are larger, they have longer and broader leaf bases, the acuminations are longer and more sharply pointed, and the costae are more longly excurrent.

As many investigators have pointed out, especially Andrews (1920), in *Weissia*, *Astomum* and *Hymenostomum*, generic distinctions are not always sharp, and the genera are connected by species which with about equal logic could be placed in either genus. For example, *W. controversa* and *W. wimmeriana* (Sendtn.) BSG. sometimes lack even rudiments of a peristome. *Astomum ludovicianum* often produces capsules with a row of differentiated cells, forming a line of demarcation between a possible urn and operculum, although the latter apparently is never functional. Also the setae, normally short and bearing immersed capsules, are sometimes exserted beyond the perichaetium. An oral membrane is only partially developed in *H. tortile* (Schwaegr.) BSG., and usually disappears by the time the capsule is mature. Finally, *H. rostellatum* (Brid.) Schimp., which is gymnostomous and has an oral membrane, is cleistocarpous and has immersed capsules, suggesting a relationship with *Astomum*. Loeske (1910) resurrected even another genus to accommodate the latter species, *Kleioweissia* Bahrh., which, as Andrews (1920) stated, makes matters worse rather than better.

Nicholson (1905) published the first account of hybrids in the *Weissia* complex. He described hybrid sporophytes from southern England, which he attributed to natural crosses between *Astomum crispum* (Hedw.) Hampe (as *Weissia*) ♀ and *Weissia fallax* Schlm. (as *W. crispata* Lindb.) ♂, and between *W. fallax* ♀ and *A. crispum* ♂. Although there were minor differences between the reciprocals, sporophytes from both crosses were intermediate between the parents. Spores from the *A. crispum* ♀ × *W. fallax* ♂ capsules were abortive and produced in small numbers, or they were brownish red, which suggests they may have been non-viable. Viable spores are generally greenish. The reciprocal

hybrid, according to Nicholson, produced no spores or at most a few undeveloped hyaline cells clustered around the columella.

A year later, Nicholson (1906) found plants in a rough, stony field near Lewes, Sussex, which, he concluded, were hybrids between *Astomum crispum* ♀ and *Hymenostomum microstomum* (Hedw.) R. Br. ♂. The hybrid sporophytes had short setae, a differentiated operculum, no peristome and portions of a membrane over the mouth. The spores were described as reddish brown, again, suggesting sterility, but attempts at germination were not mentioned. The reciprocal was not found.

An even more remarkable hybrid was reported a few years later by Nicholson (1910). On the Sussex coast, between Seaford and Eastbourne, he found capsules on plants of *Tortella flavovirens* (Bruch) Broth. (as *Trichostomum*), which he regarded as hybrids between *Tortella flavovirens* ♀ and *Astomum crispum* ♂. The capsules were stout, on very short setae, and had calyptrae which appeared too large for the capsules. Capsules were partially cleistocarpous, and the peristomes were imperfectly developed. According to Nicholson, gametophytes were clearly *T. flavovirens*, a determination he claimed was confirmed by Levier. Nicholson's account of this extraordinary hybrid caused Andrews (1920) to wonder if generic lines in this complex should be drawn even broader and possibly involve *Tortella* (C. Müll.) Limpr. and *Trichostomum* Bruch. In practice, however, Andrews never went this far.

In a later paper, Andrews (1922) himself, now aware of the possibility of hybrids in the complex, speculated that the type specimen of *Astomum nitidulum* BSG. might have been a hybrid involving *A. muhlenbergianum* (Sw.) Grout ♀ × *Weissia controversa* ♂. Apparently, the type of *A. nitidulum* consisted of a single plant, or, at most, a few plants, which were intermixed in the type collection of *A. sullivantii* BSG. (= *A. muhlenbergianum*). Unfortunately, when Schimper returned the type specimen, Sullivant lost the contents in opening the packet. Andrews based his speculation of hybridity on drawings found in the Sullivant herbarium at Harvard University and upon presumed duplicate material collected by Sullivant.

Khanna (1960*a*, *b*) found the chromosome number, n = 26, in *Astomum exserta* Broth. (as *Weissia*), from the Siwalik Range, India, and concluded that it is an amphidiploid. He claimed that *A. exserta* capsules are intermediate in all respects between *A. crispum* and *W. controversa*, and must have arisen through hybridization of these two species, followed by chromosome doubling. Meiosis in *A. exserta* was normal in all respects. He found no multivalents, disjunction was normal, and the chromosomes were distributed equally to the four nuclei. Khanna assumed that lack of irregularities in meiosis ruled out any possibility of autotetraploidy, but this is not correct (Stebbins, 1950, 1958; Lewis & John, 1963).

Reese and Lemmon (1965) described natural hybrid capsules which were discovered near Pont Brule, Lafayette Parish, Louisiana, intermixed in populations of *Astomum muhlenbergianum* (as *Weissia*), *A. ludovicianum* (as *Weissia*), and *W. controversa*. The hybrid capsules were intermediate between *A. ludovicianum* and *W. controversa*. No evidence could be found of *A.*

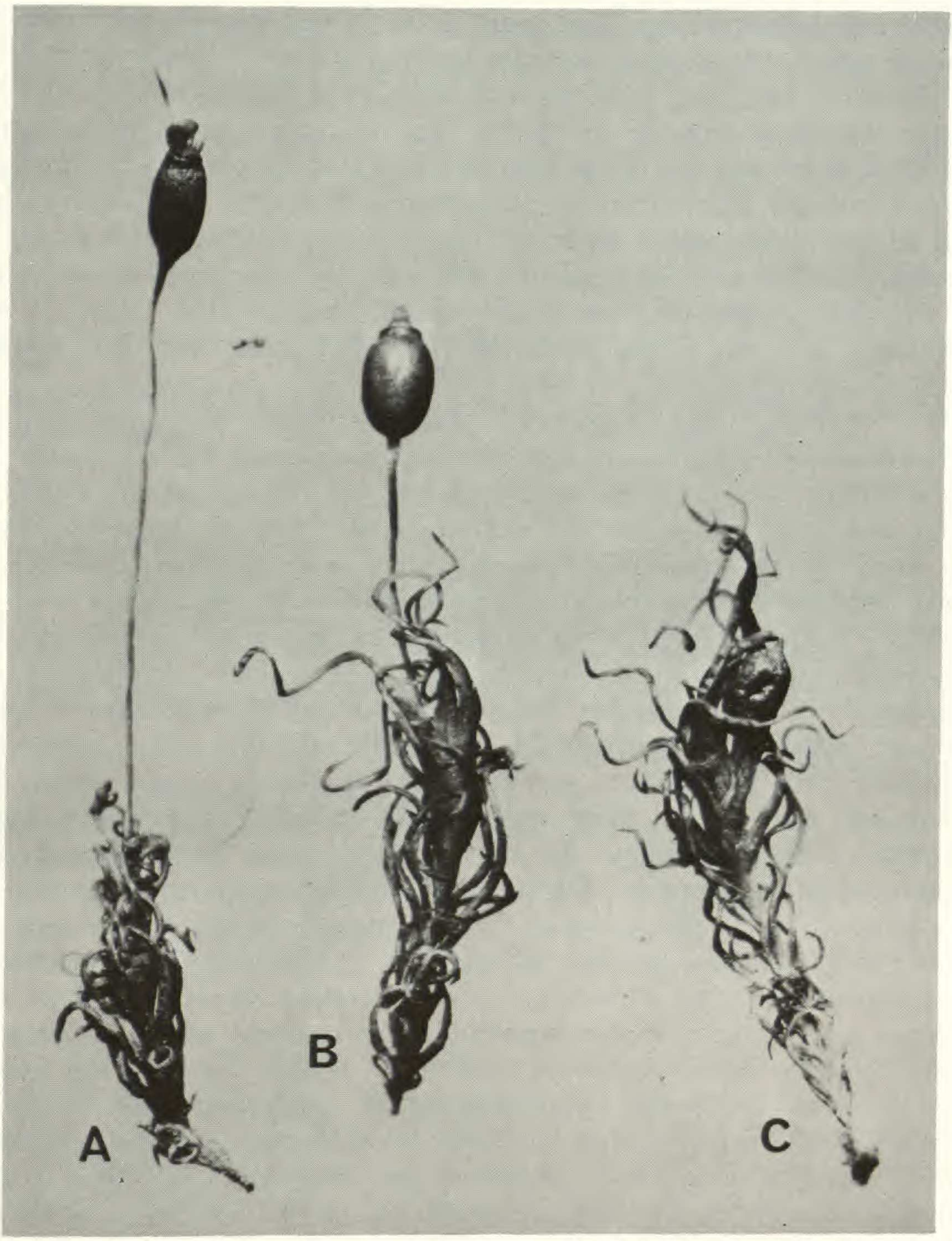


FIGURE 1. B. Hybrid sporophyte borne by female plant of *Astomum ludovicianum*. — C. Nearby plant of *A. ludovicianum*, with normal sporophyte. — A. Nearby plant of *Weissia controversa*, the paternal species. × 15.

muhlenbergianum parentage in any of the hybrids. Stating that "no authors have yet been able to demonstrate any significant or convincing differences between the gametophytes of *W. controversa* and *A. ludovicianum*," Reese and Lemmon felt that "either species may serve as male or female parent." All of the hybrid sporophytes that we have examined in herbarium specimens (*Reese* 7925; LAF, DUKE), however, are on *Astomum* gametophytes (Fig. 1). It seems probable, therefore, that the Louisiana hybrids all resulted from *A. ludovicianum* ♀ × *W. controversa* ♂.

Reese and Lemmon found that most of the hybrid capsules they examined contained spores, but in some capsules the spores had obviously aborted and were shrunken, collapsed, or arrested in tetrads. A few hybrid capsules contained no spores or only an amorphous mass of tissue around the columella. Spores from seven capsules of the hybrid were tested for viability by sowing on Benecke's nutrient agar. Viability ranged from 0 to 2%; two capsules contained all non-viable spores, while the remaining five yielded 7, 8, 8, 15, and 22 germinating spores, respectively. Attempts to grow leafy gametophytes from the protonemata of these viable hybrid spores failed (personal communication), but the failure could have resulted from less than optimum cultural conditions.

Claire Williams (1966) reported hybrid sporophytes which she attributed to *Astomum muhlenbergianum* ♀ × *Weissia controversa* ♂. They were generously intermixed with normal sporophytes of the two parents on heavy clay soils in well-established permanent pastures near Thedford, Lambton County, in southern Ontario. The hybrids were clearly intermediate between the two parents. Hybrid capsules contained spores about 22μ, but many were described as "imperfect." Attempts to germinate the hybrid spores were unsuccessful.

Among the genera, *Astomum*, *Hymenostomum* and *Weissia*, thirteen species have been investigated cytologically (Table 2). Of these, ten species have populations with the number, $n = 13$; three species have populations with the number, $n = 13 + m$; three species have polyploid populations, $n = 26$. Populations with and without the m-chromosome have been reported in *A. crispum* and *W. controversa*. Diploid and tetraploid populations, $n = 13$ and 26, are known in *H. microstomum*. These numbers are in close accord with those reported for other members of subfamily Trichostomoideae (Pottiaceae).

Chromosome numbers, based on North American populations, have been published for the three species reported to be involved in the formation of natural hybrids. Steere, Anderson, and Bryan (1954) recorded the number, $n = 13$, for two Californian populations of *W. controversa* (as *W. viridula* Hedw.), and the same number has since been found in four populations in North Carolina (Bryan, 1956; Al-Aish & Anderson, 1961) including a single population identified as var. *longiseta* (Lesq. & James) Crum, Steere & Anderson (as var. *australis*), and in single populations in Ohio (Anderson & Lemmon, 1967) and Iowa (Messmer & Lersten, 1968). The number, $n = 13 + m$, has not heretofore been recorded for North American populations of *W. controversa*.

Bryan (1956) has published the only chromosome numbers for North American species of *Astomum*. She found the number, $n = 13$, in a single population of *A. ludovicianum* and the number, $n = 26$, in two populations of *A. muhlenbergianum*; each collection came from North Carolina. She found the chromosome morphology in each species similar to *Weissia controversa*, except for the doubled number in *A. muhlenbergianum*. Meiosis was completely normal in the tetraploid, however. She observed no multivalents, and she mentioned no secondary associations of bivalents. A large bivalent, which has been described as characteristic of nearly all the species of this complex which have

TABLE 2. Summary of chromosome numbers reported for the three genera, *Astomum*, *Hymenostomum* and *Weissia*.

	Haploid number n =	Location	Reference
Astomum			
A. crispum	13	Japan	Sannomiya, 1958
	13	India	Khanna, 1959*a*, *b*; 1960*a*, *b*
	13	Hungary	Györffy, 1964
	13 + m	India	Khanna, 1960*a*, *b*
A. exserta	26	India	Khanna, 1960*a*, *b*
A. ludovicianum	13	USA	Bryan, 1956
A. muhlenbergianum	26	USA	Bryan, 1956
Hymenostomum			
H. krassavinii	13 + m	USSR	Lazarenko *et al.*, 1968
H. microstomum	13	Hungary	Györffy, 1964
	13	USSR	Visotskaya, 1967
	13	England	Smith & Newton, 1968
	13	USSR	Lazarenko *et al.*, 1969
	26	USSR	Visotskaya, 1967
H. papillosissima	13	USSR	Lazarenko, 1967
	13	USSR	Lazarenko *et al.*, 1970
H. tortile	13	USSR	Visotskaya, 1967
	13	USSR	Lazarenko *et al.*, 1969
Weissia			
W. controversa	13 + m	Finland	Vaarama, 1950
	13	USA	Steere *et al.*, 1954
	13	Japan	Sannomiya, 1955
	13	USA	Bryan, 1956
	13	India	Khanna, 1960*a*, *b*
	13	USA	Al-Aish & Anderson, 1961
	13	Hungary	Györffy, 1964
	13	USA	Anderson & Lemmon, 1967
	13	USSR	Visotskaya, 1967
	13	USSR	Lazarenko *et al.*, 1967
	13	England	Smith & Newton, 1967
	13	USA	Messmer & Lerston, 1968
	13	USSR	Lazarenko *et al.*, 1968, 1969
	13	Wales	Ramsay, 1969
var. *longiseta*	13	USA	Anderson & Bryan, 1958
var. *densifolia*	13	England	Smith & Newton, 1967
var. *edentula*	8	India	Gangulee & Chatterjee, 1960
W. fallax	13	Wales	Smith & Newton, 1967
W. longidens	13	Japan	Sannomiya, 1955
W. occidentalis	13	England	Smith & Newton, 1968
W. rutilans	13	USSR	Visotskaya, 1967

been investigated (Table 2), was noted by Bryan to be present in *A. muhlenbergianum*, but she did not indicate if it was present in duplicate, as might be expected if autopolyploidy is involved.

There are no published accounts of meiotic or other chromosome studies

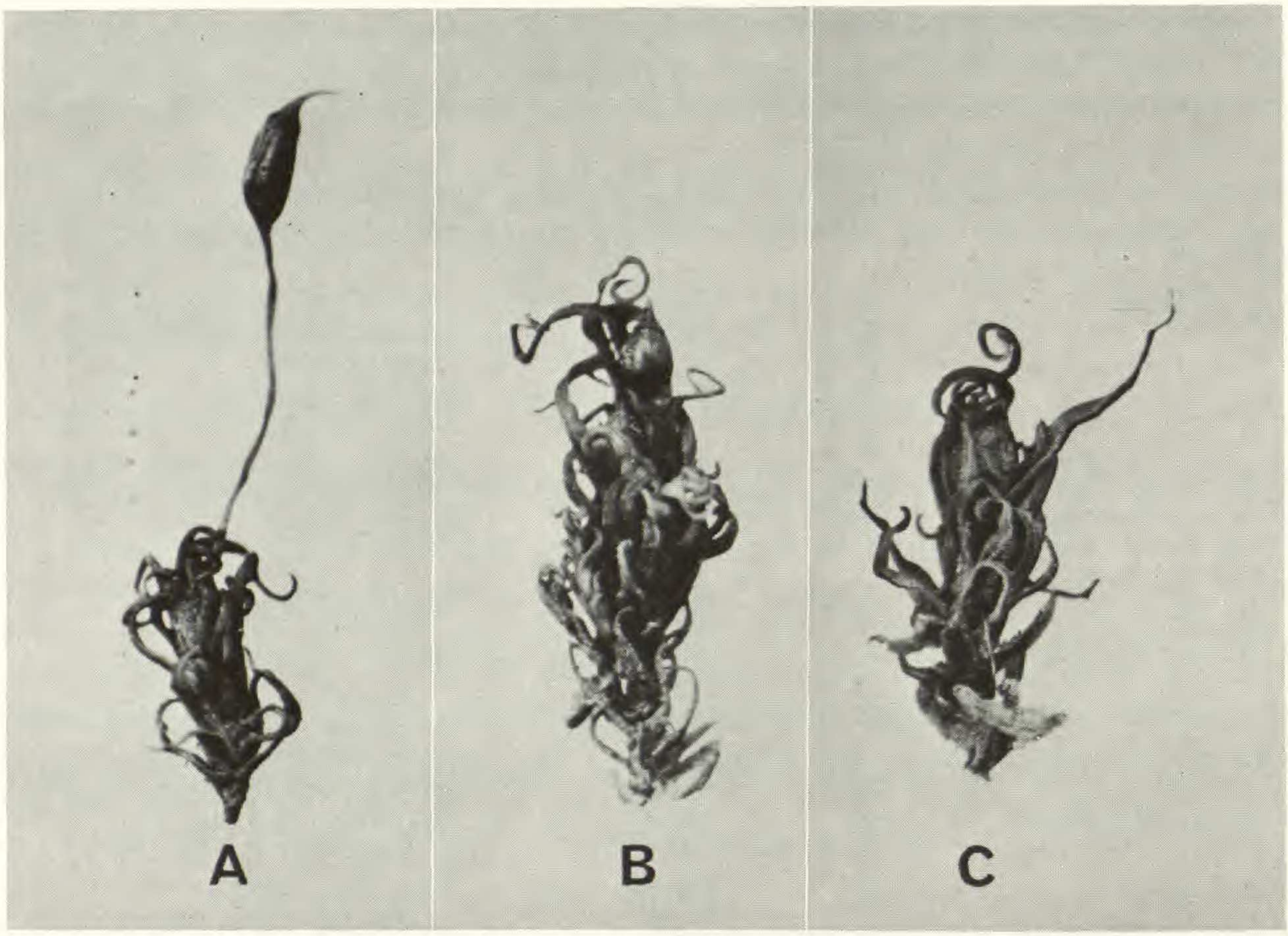


FIGURE 2. B. Hybrid sporophyte borne by female plant of *Astomum muhlenbergianum*. — C. Nearby plant of *A. muhlenbergianum*, with normal sporophyte. — A. Nearby plant of *Weissia controversa*, the paternal species. × 15.

of the *Astomum* × *Weissia* or other hybrids in this species complex. If Khanna (1960*a*, *b*) has made the correct interpretation that *A. exserta* is an amphidiploid, involving *A. crispum* × *W. controversa*, his studies include the first cytological analysis of a natural hybrid.

The present study was begun in 1966, as a joint undertaking, to investigate meiotic chromosome behavior in available natural intergeneric hybrids in *Weissia* and *Astomum*, and to examine meiosis and variations in chromosome number among different populations of the parental species. This paper presents the results of meiotic studies of two natural hybrids, *A. ludovicianum* ♀ × *W. controversa* ♂ and *A. muhlenbergianum* ♀ × *W. controversa* ♂, and a preliminary report on intraspecific chromosome variations in the species involved. Possible applications of the results to the taxonomy of the complex, specifically the status of the genus *Astomum*, and to problems of reproductive isolation are discussed.

MATERIALS AND METHODS

Weissia controversa occurs throughout North America. It is semi-weedy and is often found in disturbed areas. It is most abundant in the southern United States, becoming less and less common to the north and to the west. Tolerant of full shade to full sunlight, it is restricted to bare soils and rocks. It is never epiphytic or epixylic. Road banks, pastures, lawns, and waste places

that are sparsely vegetated are favored sites, but it is also found on bare patches of soil in woods. The species is not tolerant of high moisture, apparently, preferring xeric or mesic situations.

Astomum muhlenbergianum is confined to eastern North America, where it ranges from southern Canada to the Gulf of Mexico. *Astomum ludovicianum* is restricted to southern United States, extending north to Kentucky, West Virginia, and Maryland. The two species of *Astomum* share identical habitats with *W. controversa*, and occasionally the three species are found together.

Phenological development is approximately the same in all three species. In Florida and the Gulf States, meiosis begins in some capsules in November and continues into March, when most capsules are mature. The largest number of capsules in meiosis was found in January in the Gulf area. In the Middle Atlantic States, capsules can be found in meiosis more or less continuously from early September to June. The largest number of capsules in meiosis was found in November and in March, decreasing markedly during the colder months of December, January, and February. Across the northern United States and in the West, the meiotic season apparently lasts from March to August, being later at more northern latitudes and higher altitudes. We have few observations, however, for northern and western regions.

All of the spore mother cells of a single capsule undergo meiosis at about the same time, but there is usually considerable spacing of stages among different capsules of the same clump. In a single clump a succession of meiotic capsules may extend over a period of two months. Occasionally in the Southeast, some of the capsules in a clump will undergo meiosis in the fall, while the remaining capsules delay meiosis until spring.

There is a prodigious production of sporophytes in *Weissia controversa* and in both species of *Astomum*. Partly, this is due to the large number of archegonia, up to eight in each perichaetium, and antheridia, up to twenty in each perigonium. Only about 10% of the archegonial plants produce perigonia, but nearly every plant produces archegonia. Also, both archegonia and antheridia develop sequentially over a period of several months. During the fertile period, at any one time a perichaetium will contain from one to three receptive archegonia, and a perigonium will have usually one or sometimes two ripe antheridia.

All of the chromosome studies described here were obtained from living capsules brought into the laboratory, where the preparations were made and studied immediately. Permanent slides were not made. Mitotic preparations were not attempted.

Samples for cytological examination were collected in polyethylene bags and labeled with locality, habitat, and date of collection. They were then sealed and usually placed in an ice chest until taken to the laboratory, where they were stored at about 5°C. until they were studied. As noted by Anderson and Crum (1958), we found no effects of low temperature on meiosis except that its rate is decreased. If proper humidity conditions are maintained in the plastic containers, and if there is sufficient number of properly spaced stages of younger capsules, a succession of meiotic capsules

can be maintained in a clump for six weeks or longer. If plants are kept too moist, they will be overrun with molds and bacteria. If they are too dry, capsules shrivel and meiosis stops or becomes aberrant.

Meiosis occurs in all of the species studied after the capsule has reached its full size, and while it is still green. Capsules which have developed a slight yellowish hue and a characteristic translucence are apt to be in meiosis. Usually, when capsules become distinctly yellow and begin to look opaque, meiosis is complete. When growing in full sun, walls of the urn and rarely the beak develop considerable reddish coloration before the onset of meiosis. This obscures all external clues, making it necessary to resort to chance and trial and error.

The cytological techniques used were essentially the same as those described by Steere, Anderson and Bryan (1954) with some of the modifications added that were outlined by Anderson and Crum (1958), especially those pertaining to the handling and storing of living material. Only a brief outline of the procedures will be given here.

Promising capsules were selected, the contents squeezed out in a drop of Carnoy's solution (3:1), a drop of water, or directly into a drop of dye on a clean slide. The sporocytes were freed from the columella and any extra debris removed. If the dissection was carried out in water, as soon as the sporocytes were free, the water was removed with a piece of absorbent paper, and the fixative or dye was then applied. Dissections in the fixative were allowed to stand until most of the fixative evaporated, and then the dye was applied. Any one of these three procedures is entirely satisfactory. The dye used was acetic orcein (synthetic orcein saturated in 45% acetic acid and filtered). After applying a cover glass, the slide was heated almost to boiling several times in succession. The chromosomes were then spread and flattened by repeated tapping and by pressing on the cover glass. The progress of this operation was checked at intervals under the microscope. When the preparation was completed, the cover glass was sealed by ringing with petroleum jelly. Occasional slides were allowed to stand overnight to improve the staining.

A complete series of voucher specimens was deposited either in the herbarium of Duke University (DUKE) or the University of Southwestern Louisiana (LAF).

OBSERVATIONS

1. Polymorphism for Chromosome Numbers

Early in this study the chromosome numbers, $n = 13$ and $n = 13 + m$, were discovered in *Weissia controversa*[4], and $n = 13$, $n = 13 + m$, and $n = 26$, in both *Astomum ludovicianum* and *A. muhlenbergianum*. We addressed our-

[4] The number, $n = 26$, was discovered in a few populations in Tennessee, Missouri, and Arkansas. These plants had previously been identified as *Weissia controversa*, but they possess morphological and habitat distinctions which we think may justify separating them taxonomically. We are currently examining this question.

selves first to the question, how are the different chromosome numbers distributed within or among populations?

As Stebbins (1950) points out, the term population has no precise meaning, but it generally denotes a group of interbreeding individuals. Almost nothing is known about breeding systems in mosses. Gene flow distances are completely unknown. We can only make inferences based on knowledge of where the sex organs are produced, how they are distributed among the leafy plants, and specific growth habits of the species involved. There is even less information concerning spore dispersal and how local populations spread.

Weissia and *Astomum* are caespitose and, with few exceptions, grow in distinct, clearly delimited clumps, consisting of hundreds of more or less tightly compacted leafy gametophytes. Plants are autoicous, but only about 10% of the archegonial plants bear antheridia, a device that increases cross-fertilization within the clump. It is not known whether the clump is clonal, whether it originates from a single spore or from a vegetative propagule, whether it comes from more than one spore from the same capsule or from different capsules. These are important questions that require study.

The clump itself, however, would appear to be the interbreeding unit in these species. It is not easy to visualize movement of sperm from one clump to another, unless the clumps are contiguous. Perhaps, the moss clump corresponds to the "local population" or "deme," which Mayr (1970) defines as "a group of individuals so situated that any two of them have equal probability of mating with each other and producing offspring." At any rate, we have chosen to use the clump as the population unit, and we shall use the terms clump and population interchangeably. We realize this is a narrow application of the term population. Some might wish to use sub-population or an even lesser designation. But, if we expand the meaning of population beyond the individual clump, we have no notion where to stop. The clump comprises a discrete group of interbreeding individuals and, until we know in mosses how much comprises what Mayr (1970) terms "the community of potentially interbreeding individuals at a locality," it is a practical unit for study. If two or more clumps were found overlapping, they were regarded as a single population in this study.

To determine whether individual clumps contained plants with different chromosome numbers, a series of sampling studies were carried out with *Weissia controversa*, with populations from four areas. At each locality, from four to six individual clumps were taken from an area of approximately 100 m × 10 m. All of the habitats were road banks, along which clumps were distributed, usually scattered in bare spots among grasses. Originally, the intent was to sample 20 capsules from each clump. The number was soon dropped to 10, because of the work involved and because not every clump yielded 20 capsules in meiosis. Even a goal of 10 was unattainable in several clumps. The results are shown in Table 3.

The data in Table 3 indicate there is a high degree of uniformity in chromosome number within individual populations. This inference is strengthened by the fact that we have also examined from 3 to 5 capsules from hundreds of

TABLE 3. Chromosome numbers of sampled capsules from individual populations from four different areas. Collection numbers are those of Lewis E. Anderson, and specimens are on file in the Duke Herbarium.

Locality	Date	Collection Number	No. Capsules Examined	Chromosome Number	
				n = 13	n = 13 + m
Durham, N. C.	12/'67	20,212	12	12	
		20,213	15		15
		20,214	19	19	
		20,215	11	11	
		20,216	20	20	
		20,217	11		11
Washington, Alabama	12/'68	20,457	8	8	
	12/'68	20,458	10	10	
		20,459	10	10	
		20,460	6	6	
		20,461	10	10	
		20,462	4	4	
Live Oak, Florida	1/'70	20,763	10	10	
		20,767	10		10
		20,768	8	8	
		20,769	9	9	
Mayo, Florida	1/'71	21,009	10		10
		21,010	10	10	
		21,011	10	10	
		21,012	9		9
		21,013	6	6	

populations of *Weissia controversa*, distributed over an even wider geographical area, and we have not encountered a single polymorphic population. Nothing short of determining the chromosome number of every plant in a clump can establish beyond doubt that a population contains only a single number, but if mixed populations exist they are either rare or the frequencies of plants with supernumerary chromosomes are so low they are difficult to demonstrate through sampling.

The question of whether individual populations are clonal is still not answered. This will have to be studied experimentally, hopefully in the field under natural conditions. The data do indicate, however, that there is limited mixing of propagules during dispersal or else protonema from a single propagule gains dominance and crowds out competitors. The former seems more likely.

2. Non-random Distribution of Supernumerary m-chromosome Populations

Preliminary observations indicated that populations of *Weissia controversa* with m-chromosomes are not distributed at random. This prompted us to expand the studies geographically and to sample as many populations as practicable over as large an area as we could manage. Between 1966 and 1969, we obtained counts from 460 populations of *W. controversa*, 17 of *Astomum ludovicianum*, and 10 of *A. muhlenbergianum*. The scarcity of collections of the two species of *Astomum* reflect not only their infrequent occurrences,

TABLE 4. Distribution of cytological collections of *Weissia controversa*, by states, and the number and percentages of n = 13 populations and 13 + m populations in each state.

State	Total Number of Collections	Chromosome Number			
		n = 13		n = 13 + m	
		Number of Collections	%	Number of Collections	%
Alabama	58	47	82	11	18
Arkansas	16	14	87	2	13
Florida	33	27	82	6	18
Georgia	72	67	93	5	7
Louisiana	70	59	80	11	20
Mississippi	39	30	77	9	23
North Carolina	78	59	76	19	24
South Carolina	35	25	72	10	28
Tennessee	29	29	100	0	0
Virginia	12	12	100	0	0
Others	18	17	–	1	–
Totals	460	386	84	74	16

but their camouflaged habits, as well. Collections of the three species were distributed over 17 states and 2 provinces of Canada, but the collections were mainly centered in 10 southern and southeastern states. Much of the collecting was carried out fortuitously while both of us were travelling on other business, and some collections were shipped to us by colleagues. Sampling, therefore, was not always by design, and much of it was carried out along road banks of arterial highways.

Since the bulk of the data referring to the distribution of chromosome numbers involve populations of *Weissia controversa*, we have segregated the data for this species. The results are compiled in Table 4, arranged by states, and include frequencies of chromosome numbers for each state. States for which we have fewer than 10 collections are combined.

The 13-chromosome populations comprised 386 or about 84% of the clumps examined, while populations with the number, n = 13 + m, totalled 74 or about 16%. Excepting Georgia, whose low m-frequency is due to a strong bias in collecting in mountains and Piedmont, the differences in frequencies of the m-chromosome-populations from state to state are not very striking.

If the population samples are grouped according to physiographic province, however, a strong non-random pattern is revealed. The results are shown in Table 5. Ozarks, Cumberlands, and Appalachians are grouped together as Mountains.

The m-chromosome frequency is approximately 23% in the Atlantic Coastal Plain, in contrast to only 12% in the Piedmont Plateau and less than 3% in the Mountain Provinces. It should be pointed out that we have not studied any populations in the Coastal Plain north of North Carolina.

Not enough populations of either species of *Astomum* were studied to

TABLE 5. Distribution of m-chromosome populations according to physiographic province.

Physiographic Province	Total Number of Collections	Chromosome Number			
		n = 13		n = 13 + m	
		Number of Collections	%	Number of Collections	%
Coastal Plain	254	197	77	57	23
Piedmont	118	104	88	14	12
Mountains	75	73	97	2	3

establish reliable frequencies for the different chromosome populations. Seventeen populations of *A. ludovicianum* were studied. Eleven had the number, n = 13 (6 in Louisiana, 3 in North Carolina, 1 in Florida, and 1 in Kentucky), while three had the number, n = 13 + m (all from Louisiana). In addition, two populations (one each from Mississippi and Georgia) were tetraploid, n = 26.

Ten populations of *Astomum muhlenbergianum* were studied. Seven had the number, n = 13 (3 in North Carolina, 2 in Louisiana, and 1 each in Kentucky and Tennessee). A single population with the number, n = 13 + m, was discovered in Louisiana, while two tetraploid populations, n = 26, were found in North Carolina. Bryan (1956) also found two tetraploid populations of this species in North Carolina.

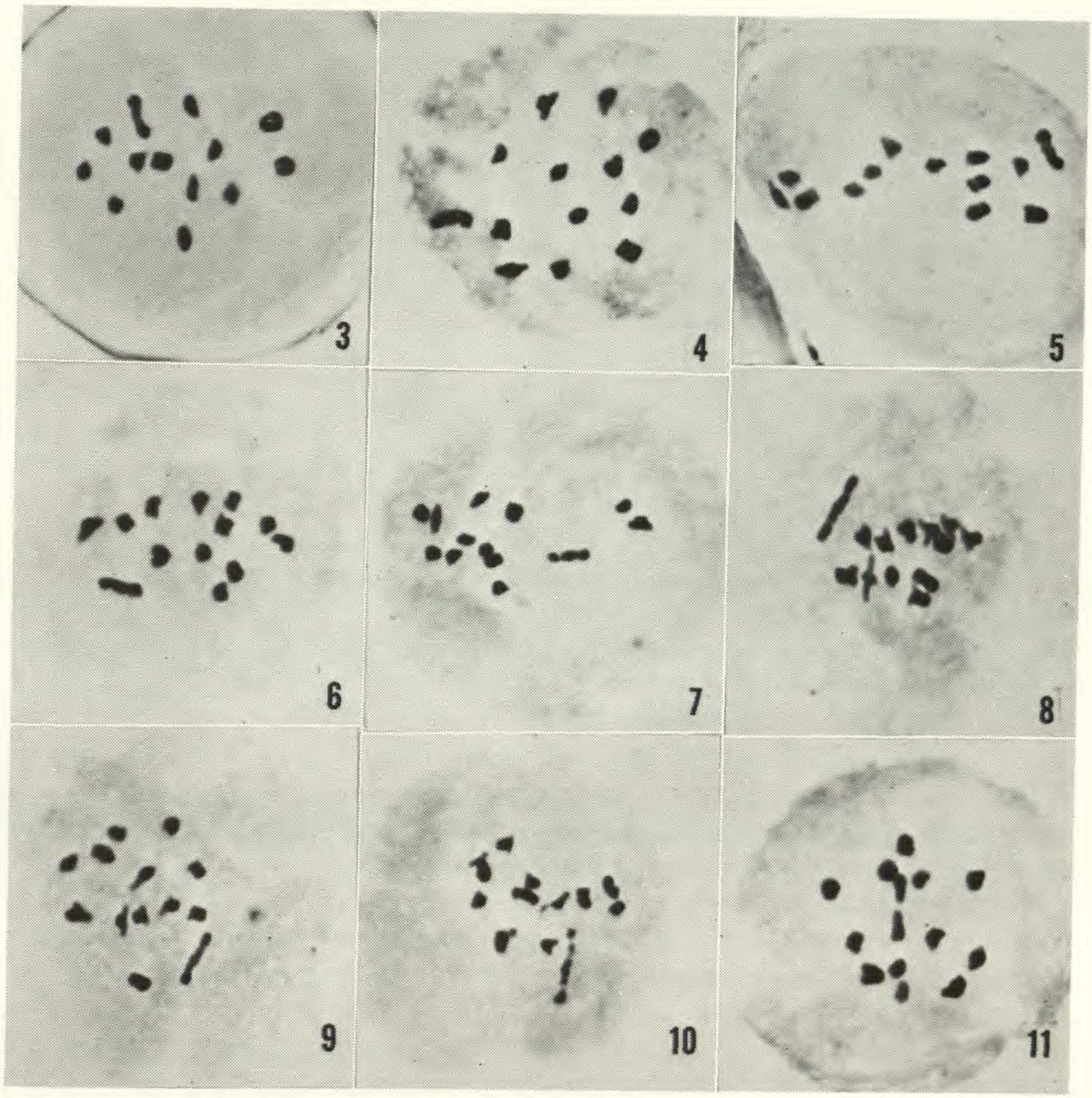
3. The Standard Meiotic Complement

The standard chromosome complement is similar, if not identical, in the three species, *Weissia controversa* (Figs. 3–11), *Astomum ludovicianum* (Figs. 21–23), and *A. muhlenbergianum* (Figs. 30–32). The number, n = 13, is regarded as the standard complement, not only in this complex, but probably in all of the sub-family Trichostomoideae.

The complement is characterized by the presence of a conspicuous, rod-shaped bivalent (Figs. 3–10, 21–22), which is about 2 μ long when completely condensed. It is held together by a single terminal chiasma, and has a primary sub-median kinetochore and a sub-terminal secondary constriction (Fig. 10). During prometaphase the large rod-shaped bivalent undergoes pronounced stretching (Figs. 8–10), and nearly always disjoins earlier than the other bivalents.

The remainder of the complement consists of twelve even-sized bivalents, averaging about 1 μ long. None of the individuals in this group of small bivalents could be recognized from cell to cell. Kinetochore positions were not established.

Except for the large bivalent, it was not possible to estimate chiasma frequencies. Diplotene and diakinesis are rarely observed in any moss, and *Weissia* and *Astomum* are not exceptional. When a rare sporocyte was encountered in diplotene or diakinesis, the chromosomes were so clumped and so poorly delineated that it was not possible to analyze the configurations.



FIGURES 3–11. Meiotic chromosomes of *Weissia controversa*, n = 13, the standard complement. — 3–7. Polar views of prometaphase figures from North Carolina, Louisiana, Kentucky, Ohio, and British Columbia, respectively, showing uniformity in meiotic complements. — 8–10. Prometaphase, showing degrees of stretching of the large, rod-shaped bivalent. — 11. Prometaphase, showing early disjunction of the largest chromosome. × 1905.

Of possible significance, were the observations in occasional sporocytes of *Weissia controversa*, and in a single sporocyte of *Astomum ludovicianum*, in which there was obvious secondary pairing among ten of the twelve smaller bivalents. In Figure 23, from a population of *A. ludovicianum*, from North Carolina, five pairs of bivalents are secondarily associated. Three pairs are in tight association, while two pairs are more loosely paired. The large, rod-shaped bivalent and two of the smaller bivalents are unassociated. In each observed instance of secondary pairing, ten bivalents, forming five secondary pairs, were always involved. Whether they are the same bivalents in each case could not be determined, since the smaller bivalents are unidentifiable.

These observations are pertinent if a lower basic chromosome number for the complex is considered. Gangulee and Chatterjee (1960) reported the number, n = 8, in a population of *Weissia controversa* var. *edentula* (Mitt.) Chen, from India, which, although the count needs reconfirming, is indicative of a lower basic number. If the five secondary pairs which we observed in *W. controversa* are duplications, then five of these bivalents added to the three unassociated bivalents makes a total of 8, which is the number reported by Gangulee and Chatterjee in the Indian variety. This is a tantalizing parallel, which may not be coincidental.

4. The Supernumerary m-chromosome

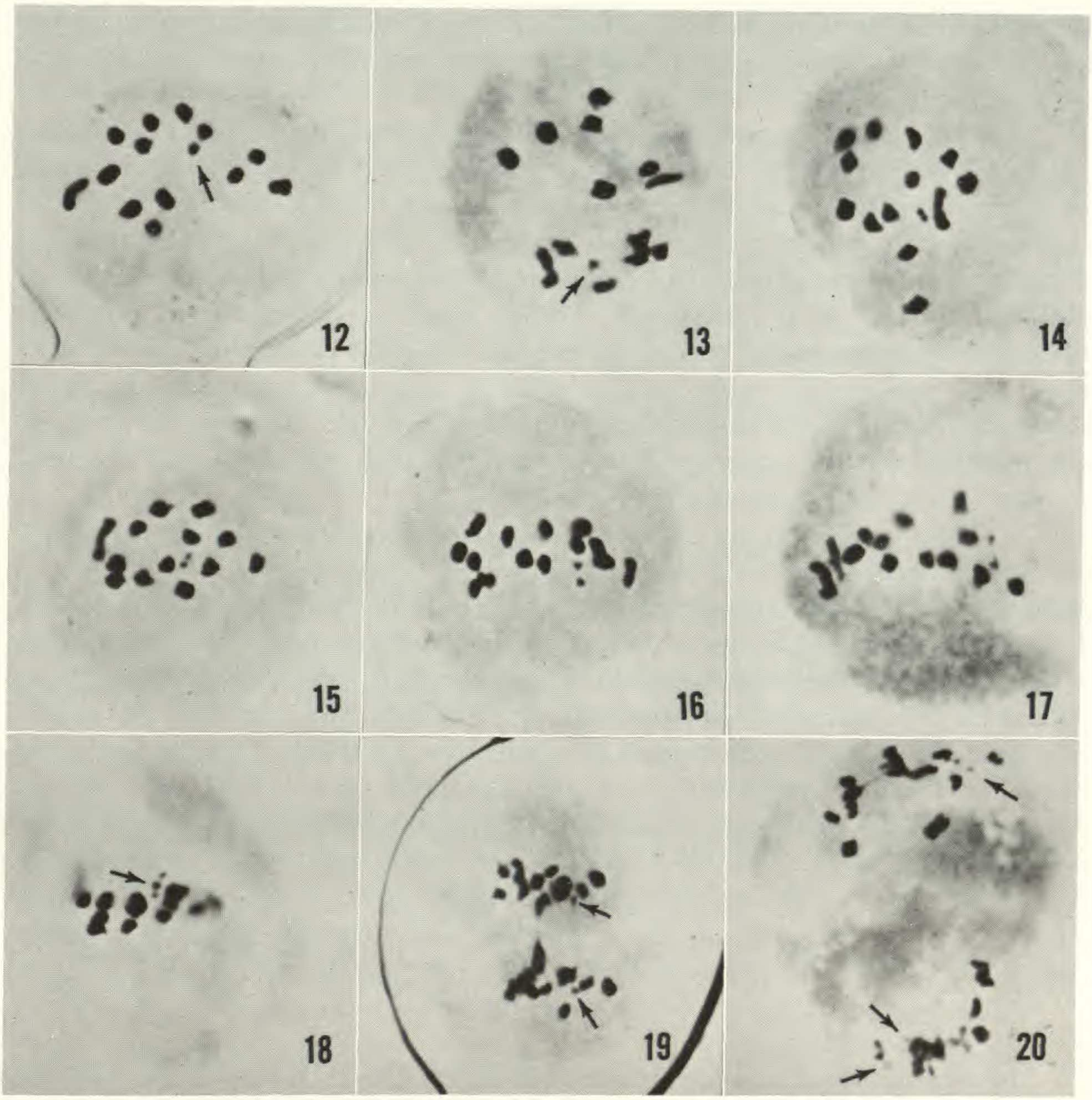
We discovered m-chromosome populations in all three species, *Weissia controversa* (Figs. 12–20), *Astomum ludovicianum* (Figs. 24–26), *A. muhlenbergianum* (Figs. 33–35), and in the natural hybrid *A. ludovicianum* ♀ × *W. controversa* ♂ (Figs. 39–42). No differences in form or behavior could be detected among the m-chromosomes of the three species or the hybrid. The descriptions that follow, therefore, apply equally to the three species, and references will be made interchangeably to figures of all the species and the hybrid.

The supernumerary chromosome observed matches the m-chromosomes which have been described in the literature for a broad range of moss species (Heitz, 1927; Vaarama, 1950, 1953; Steere, Anderson & Bryan, 1954; Bryan, 1955; Yano, 1957*a–c*, as h-chromosomes; Anderson & Bryan, 1958; Anderson, 1964; Smith & Newton, 1967; and others). From the descriptions of these investigators, m-chromosomes can be characterized as follows: They are heteropycnotic in mitotic and meiotic interphases and during prophase I of meiosis; they are largely heterochromatic; they often stain less intensely with orcein and carmine at metaphase I; rarely, they are negatively heteropycnotic; they assume a complex variety of configurations and numbers at prometaphase and early metaphase I in different sporocytes of the same capsule.

Yano (1957*a–c*) and Inoue (1968, 1969), among others, have established that the usual m-chromosome complement in gametophytic tissues of mosses consists of one small, heteropycnotic chromosome. The complement for sporophytic tissue has been shown by many investigators to consist of two m-chromosomes. Both *Weissia* and *Astomum* are autoicous, and the populations or clumps are probably mostly clonal. Thus, inbreeding is the rule. These facts, coupled with observations that m-chromosomes are stable in populations that have been watched over a period of years, strongly indicate that the diploid sporophytic m-pairs are homologous. They pair physically during meiosis, forming bivalents. Being heteropycnotic during prophase I, however, would preclude the formation of chiasmata. The bivalent configurations, therefore, are assumed to be achiasmic.

Within a single capsule, four different numbers and configurations of m-chromosomes were observed at prometaphase or early metaphase I. They will be described separately.

(1) The m-chromosomes are united into a single, more or less tightly



FIGURES 12–20. Meiotic chromosomes of *Weissia controversa*, n = 13 + m, the supernumerary complement. — 12–13. Prometaphases, each showing 13 bivalents plus the tightly associated achiasmic, m-chromosome. — 14–17. Prometaphase figures, showing distance association of the two homologous m-chromosomes. — 18. Early metaphase I, with tripartite configuration of m-chromosome, composed of a half-bivalent and two chromatids. — 19. Anaphase I, showing a single m-half-bivalent near each pole. — 20. Late anaphase I, showing two m-chromatids at each pole. × 1905.

associated bivalent (Figs. 12–13, 24, 33–34, 39). Sporophytes with this configuration present a straightforward appearance of 13 regular bivalents plus the m-bivalent. This basic configuration, which is repeated in nearly all mosses with m-chromosomes, has provided the rationale for the customary notation, n = 13 + m.

(2) The two m-chromosomes are not physically paired (Figs. 14–17, 19, 35). The two presumed homologs may be almost touching (Fig. 14) or they may be separated by varying distances (Figs. 15–17, 35). A distance-pairing relationship, however, is always evident. At anaphase I, the two homologous

elements move to opposite poles (Fig. 19), each chromosome divides during second division, and the four m-chromosomes are distributed one to each of the tetrad nuclei.

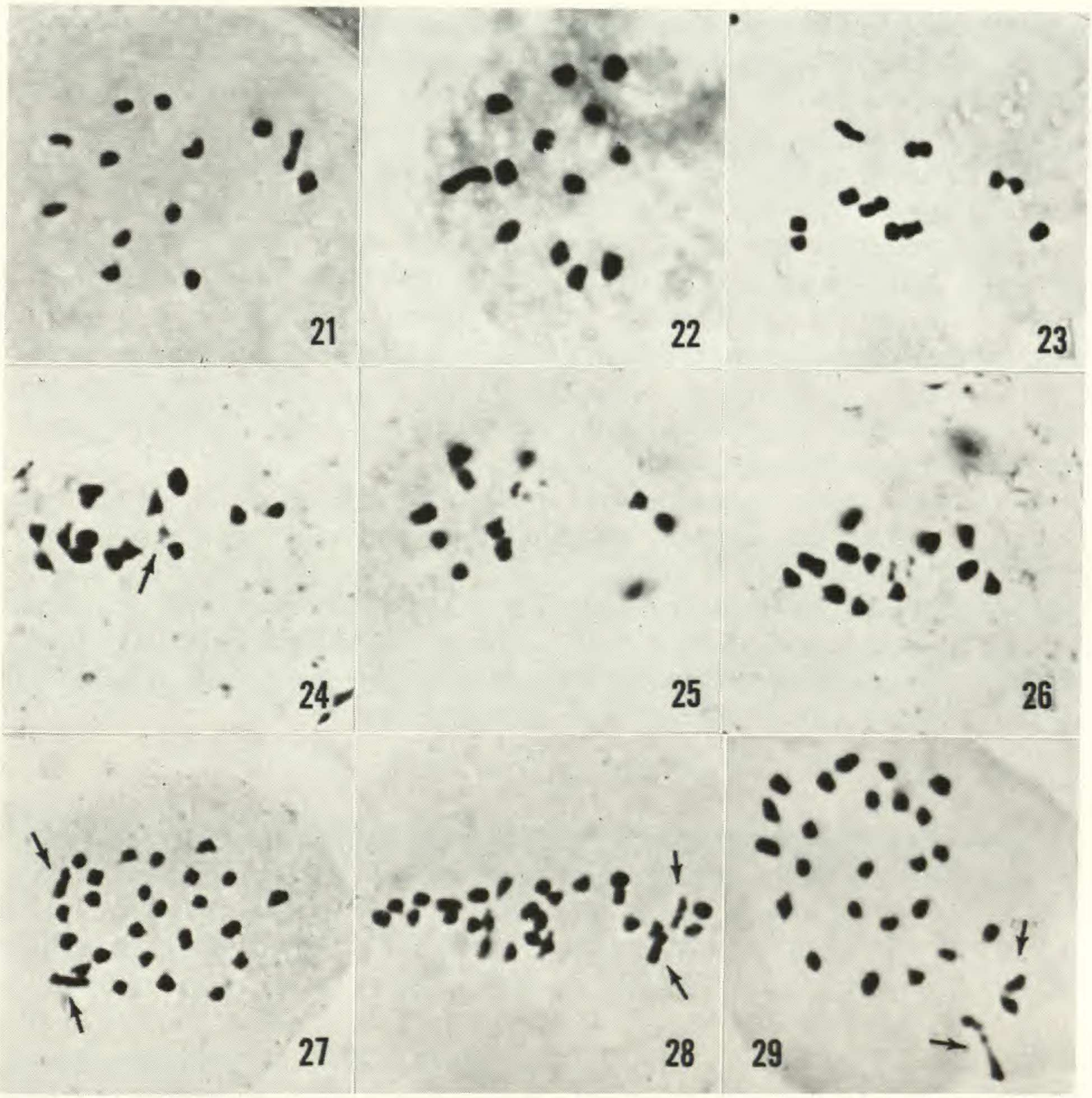
(3) The m-chromosomes are in a heteromorphic, tripartite configuration (Figs. 18, 25, 40). One of the three chromosomal elements is approximately twice as large as the two smaller elements. This is especially evident in Figure 25. Obviously, this configuration results from the division of one of the two distantly associated chromosomes described in (2). Tripartite associations were observed as early as prometaphase. During anaphase I, the single undivided m-chromosome moves to one pole, and the divided chromatids move independently but synchronously to the opposite pole (Fig. 42). In the latter figure, the two divided chromatids are at the lower pole; the undivided single chromosome is at the upper pole, where unfortunately it is somewhat obscured because it lies over another chromosome.

(4) The m-chromosomes are in a quadripartite configuration (Figs. 26, 41). This configuration was least commonly observed. It results from the division of both of the distantly associated chromosomes described in (2). Thus, the four chromosomal elements of the quadripartite association are chromatids, one of which is destined for each of the eventual tetrad nuclei. In Figure 41, which is a prometaphase sporocyte, at 9 o'clock there are four chromatids arranged in two's. The lower pair is slightly out of focus. Usually, each pair of chromatids is separated by fine chromosomal fibers (Fig. 26). During metaphase I and anaphase I, each of the four chromatids orients independently but as pairs, one pair moving to one pole the other pair to the opposite pole. In Figure 20, a pair of separated chromatids can be seen at each pole (arrows). The chromatids in the lower anaphase group of the photograph are slightly out of focus, which explains why they appear lighter in color.

At least three interpretations can be invoked to explain the variability in m-chromosome configurations described above.

The first interpretation assumes that the achiasmic m-bivalent, as seen, for example in Figures 12 and 13, undergoes desynapsis (Fig. 14) during prometaphase, forming two homologous half-bivalents (Figs. 15–17). According to this interpretation, each half-bivalent orients syntelically at metaphase I, or di-syntelically (co-orientation) with respect to each other, and moves to opposite poles during anaphase I (Fig. 19). Each half-bivalent then orients amphitelically (auto-orients), and an m-chromatid is distributed to each tetrad nucleus.

Based on this interpretation, variations in numbers of m-chromosome elements are explained in the following ways. Tripartite configurations represent desynapsed half-bivalents in which one of the half-bivalents has divided into its two sister chromatids and the homologous half-bivalent remains undivided, which explains the larger size of the latter (see Fig. 25). The undivided half-bivalent then orients syntelically, and the distantly associated sister chromatids orient independently and syntelically with respect to each other. The divided sisters and the undivided half-bivalent, however, orient di-syntelically with respect to each other, and in anaphase I, the undivided half-bivalent moves to



FIGURES 21–29. Prometaphase and metaphase meiotic figures of *Astomum ludovicianum*, showing different chromosome numbers. — 21–22. The standard complement, n = 13. — 23. Prometaphase of the standard complement, in which ten bivalents are secondarily paired. — 24–26. The supernumerary complement, n = 13 + m, showing respectively a bivalent m-configuration, a tripartite and a quadripartite m-chromosome association. — 27–29. Tetraploid meiotic figures, showing the duplicated long chromosome (arrows), and in Fig. 29, one of the long chromosomes is undergoing prometaphase stretching. × 1905

one pole, and the two sisters move together to the opposite pole (Fig. 42). Quadripartite configurations originate similarly, except that both half-bivalents divide into their respective sister chromatids (Figs. 26, 41). Thus, the two pairs of distantly associated sister chromatids move independently to the equator and the two pairs of sister chromatids orient di-syntelically, and move to opposite poles. The various configurations according to this interpretation therefore represent different degrees of disassociation of an achiasmic bivalent into two half-bivalents, one half-bivalent and two sister chromatids, or four sister chromatids.

A second interpretation assumes that both asynapsis and synapsis occur in different sporocytes. Thus, in configurations with distantly associated m-chromosomes (Figs. 16, 17, 35, for example), the latter could be half-bivalents or univalents, depending upon whether they had previously synapsed and have since desynapsed or whether they originally paired distantly and have never been in contact. This can not be determined with fixed preparations.

A third interpretation which cannot be ruled out is that in some or all sporocytes, post-reduction takes place. This interpretation assumes that an m-bivalent divides into half-bivalents, each of which is composed of non-sister chromatids. Segregation is postponed until second division. If post-reduction occurs, tripartite configurations (Figs. 18, 25, 40, 42) consist of a half-bivalent and two non-sister chromatids, while quadripartites (Figs. 20, 26) comprise two pairs of non-sister chromatids.

In summary, since m-bivalents are present throughout all of the material studied, many of the distantly associated m-chromosomes observed at prometaphase and metaphase I must comprise two desynapsed half-bivalents. It cannot be proved, however, that some are not distantly associated univalents. The designation, $n = 13 + 1mII/2mI$, might be more appropriate for populations containing the m-chromosomes. It says the population contains 13 regular bivalents plus 1 m-bivalent or 2 m-univalents.

5. The Tetraploid Complement

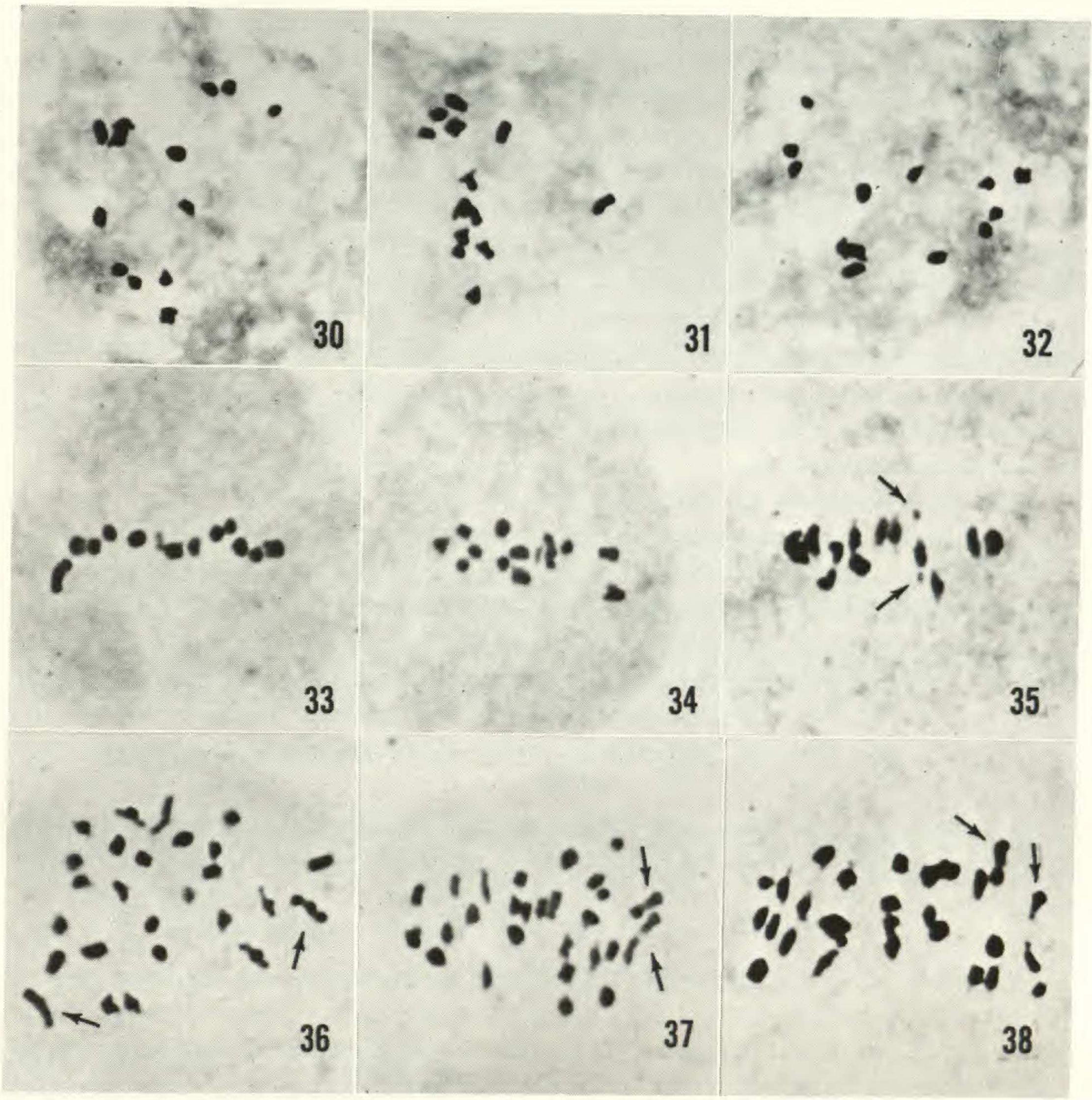
Single tetraploid populations of *Astomum ludovicianum* were encountered in Georgia and Louisiana. These are the first records of tetraploids in this species. Figures 27 to 29 are from the Georgia population. Two tetraploid populations of *A. muhlenbergianum* (Figs. 36–38) were found in North Carolina, where Bryan (1956) also found two tetraploid populations of the same species.

As in the diploids, the tetraploid complement is outwardly identical in the two species. As expected, the large rod-shaped bivalent described in the diploids, is in duplicate (Figs. 27–28, 36–37), as are the 12 indistinguishable, smaller bivalents. Respective sizes of bivalents are about the same in diploids and tetraploids. The large bivalents average about 2 μ in the tetraploids and the smaller about 1 μ.

At least one of the large bivalents undergoes prometaphase stretching (Fig. 29). In all of the tetraploid sporocytes observed, the two large bivalents disjoined asynchronously. In Figure 37, one of the large bivalents has almost separated, while its duplicate has scarcely begun anaphasic stretching. Similarly, in Figure 38, one of the large rods has completely disjoined, while the other is still tightly condensed.

Meiotic divisions in the tetraploids are completely regular, as far as we could determine. We observed no multivalents, no increase in lagging anaphase chromosomes over that observed in the diploids, no chromatin bridges, and the incidence of chromosomes which failed to get included in nuclei was no higher in the tetraploids than in the diploids.

Dr. Richard Zander kindly tested the viabilities of spores from three capsules from a tetraploid population of *Astomum muhlenbergianum*, from North Carolina,



FIGURES 30–38. Meiotic chromosomes of *Astomum muhlenbergianum*. — 30–32. The standard complement, n = 13. — 33–35. The supernumerary complement, n = 13 + m. — 36–38. The tetraploid complement, n = 26; note prometaphase stretching of the large bivalent in Fig. 37, and early disjunction of a similar bivalent in Fig. 38. × 1905

by sowing them on separate agar plates (Benecke's). He obtained 92%, 94%, and 89% germination from the three capsules. Spores from a diploid population similarly sown were, unfortunately, overrun by molds. Reese and Lemmon (1965), however, obtained 93% germination in a diploid population of *A. ludovicianum*, from Louisiana.

6. Morphological Comparisons of Populations with Different Chromosome Numbers

To determine if phenotypic differences are associated with different chromosome numbers, morphological comparisons were made. Randomly selected plants from each population were selected, washed in water and mounted on a slide

in Hoyer's mounting medium. Each plant was scored for the following: total length of plant (gametophyte and sporophyte), length of old gametophyte, length of previous years gametophyte, length of leaf, width of leaf, length of the flattened leaf base, average cell size near the leaf tip, shape of leaf apex, degree of involution of leaf margin, total length of sporophyte, length of seta, length of vaginula, total length of capsule, length of urn, width of urn, length of operculum, annulus, peristome teeth, average size of exothecial cells, degree of thickening of exothecial cells, and size of mature spores.

Weissia controversa: Forty populations each with the number, $n = 13 + m$ and $n = 13$, were sampled. Attempts were made to select one population with each chromosome number from the same site, or at least from the same general area. The forty paired populations were distributed from Louisiana to North Carolina.

The same extremes of variations for all twenty characters were found in both chromosome populations. All characters were assigned quantitative values, and each character was plotted on graph paper to show numbers of populations with and without m-chromosomes for each value. The curves for the two chromosome numbers matched almost exactly, in all 20 characters. Morphological variations between the two chromosome populations are not significant.

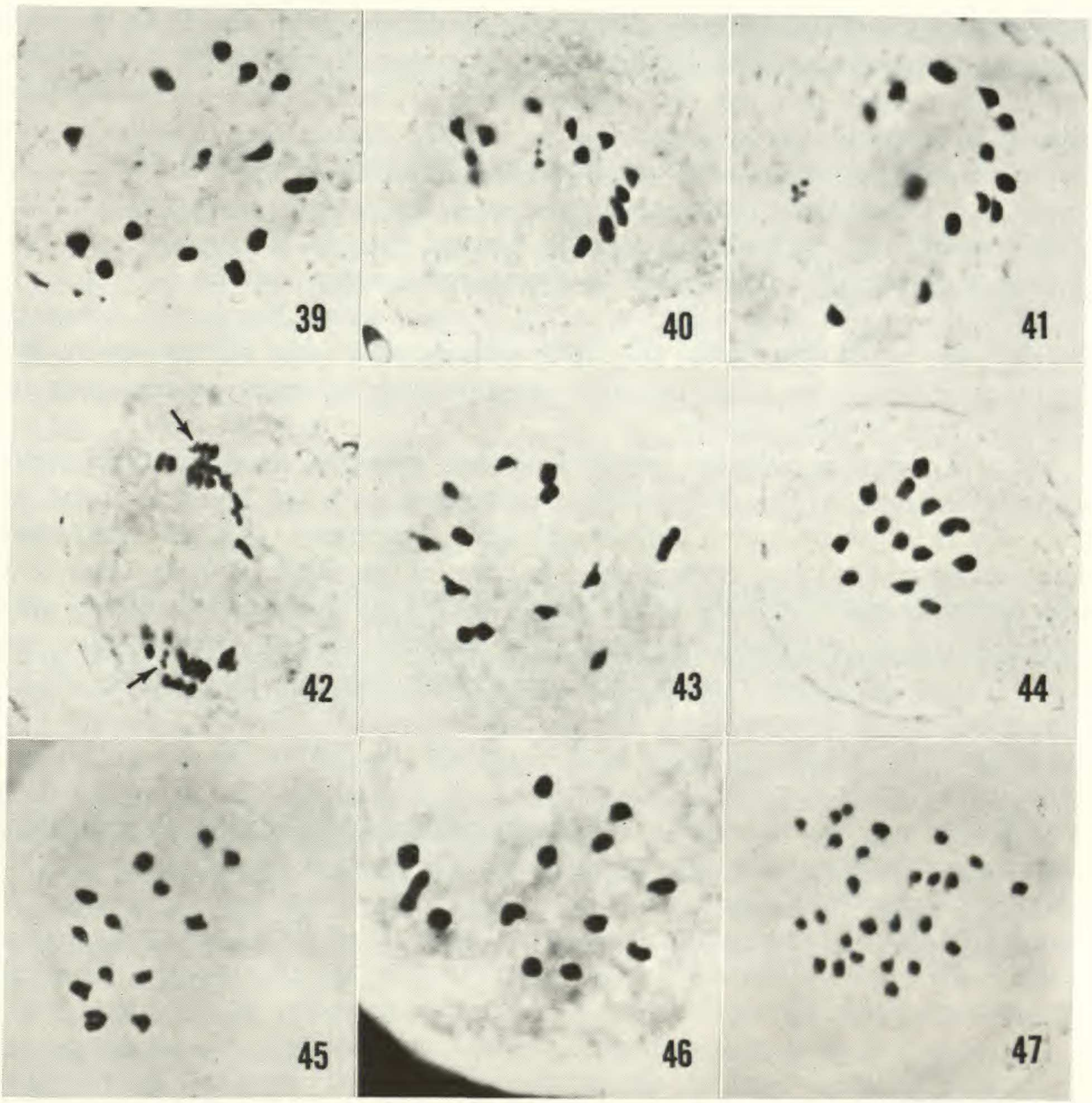
Similarly, the three chromosome races of both *Astomum ludovicianum* and *A. muhlenbergianum* were analyzed, using the same 20 morphological characters. No differences could be found between the three chromosome numbers of either species. Cell sizes and spore measurements did not average larger in the tetraploids than in the diploids, as might have been expected.

7. *Astomum ludovicianum* ♀ × *Weissia controversa* ♂

A swarm of hybrid sporophytes of this cross was discovered on clayey, poorly drained soil on a land fill about five years old on the east bank of the Vermilion River, near Lafayette, Lafayette Parish, Louisiana, on February 15, 1966 (*Betty E. Lemmon 1340*, LAF, DUKE). Meiotic stages were plentiful in hybrid capsules and in intermixed capsules of both parental species.

Plants of the parental species and hybrid sporophyte, which were growing together in a small clump, are illustrated in Figure 1. The photograph shows the male parental species, *Weissia controversa* (A), the female parental species, *Astomum ludovicianum* (C), and the hybrid sporophyte attached to a gametophyte of *A. ludovicianum* (B). These hybrids are identical, as far as we can determine, with those described by Reese and Lemmon (1965). The two hybrid localities are only 8 miles apart.

The contrasting gametophytes of *Weissia controversa* and *Astomum ludovicianum* are shown in Figure 1A and 1C. The latter has long spirally twisted leaves, with a broad, long, semi-sheathing base, and long attenuated leaf tips, while the former has leaves which are shorter, the bases are scarcely sheathing, and the leaf tips are shorter and more tightly crisped. The plants were photographed in a dried condition. It is apparent from the photograph, without having to resort to microscopic characters, that the gametophyte supporting the hybrid capsule in Figure 1B is *Astomum*, and not *Weissia*.



FIGURES 39–47. — 39–42. Meiotic chromosomes of the hybrid, *A. ludovicianum* × *W. controversa*; note absence of univalents in the standard complement. — 39. Prometaphase, with 13 tightly associated regular bivalents and one m-bivalent. — 40. Tripartite m-configuration. — 41. Quadripartite m-configuration. — 42. Late anaphase I, showing two m-chromatids at the lower pole and an undivided half-bivalent at the upper pole. — 43. Meiotic complement of *A. muhlenbergianum*, n = 13, maternal species of the Ontario hybrids. — 44. Meiotic complement of *W. controversa*, n = 13, paternal species of the Ontario hybrids. — 45–47. Meiotic chromosomes of the hybrid, *A. muhlenbergianum* × *W. controversa*, from southern Ontario. — 45–46. Prometaphase figures, showing 13 tightly associated bivalents. — 47. Early anaphase I, showing 13 half-bivalents during poleward movement; note regularity of separation. × 1905.

As can be seen in Figure 1A, the seta of *Weissia controversa* is long; the capsule is more or less cylindrical; the operculum is dehiscent; and the lid has a long, rostrate beak. In the photograph, the operculum is partially detached, revealing a well-developed peristome. The seta of the female parent, *Astomum ludovicianum* (Fig. 1C), is short, so that the capsule is immersed within the perichaetial leaves; the capsule is ovoid, the operculum is poorly

differentiated and indehiscent; there is no peristome; and the beak is short and somewhat obtuse. The hybrid capsule, as seen in Figure 1B, is partially raised on a short seta (in this respect, there is considerable variation from hybrid to hybrid; the seta pictured is slightly longer than average); the capsule is oval-cylindrical; the operculum is differentiated, but still indehiscent; there is a rudimentary peristome inside; and the beak is longer than in *A. ludovicianum* but much shorter and less pointed than in *W. controversa.* The hybrid is described in more detail by Reese and Lemmon (1965).

Hybrid capsules contained few or no spores, which Reese and Lemmon (1965) also noted, but unlike the latter authors, who were able to obtain 2% viability of spores in some capsules, we obtained no spore germinations in viability tests of four capsules.

The chromosome number, $n = 13 + m$, was observed in all of the hybrid capsules examined (Fig. 39), which corresponded with the numbers found in capsules of adjacent parent species (*Weissia controversa*, Fig. 12; *Astomum ludovicianum*, Fig. 24). Surprisingly, in view of the high abortion rates and the almost complete sterility of the spores in the hybrid capsules, meiosis was observed to be completely regular. The thirteen regular chromosomes are totally compatible and formed tight, apparently chiasmic bivalents in all of the hybrid sporocytes examined. The m-chromosomes exhibited the same variable configurations that have already been described. In Figure 39, the m-chromosomes are closely associated as a bivalent; in Figure 40, one m-half-bivalent has divided into two chromatids and the homologous half-bivalent is undivided, forming a tripartite configuration, which has already been described. In Figure 41, a quadripartite configuration of four chromatids is apparent. Disjunction is regular in the bivalents, and anaphase separation is no different in the hybrid than in the parents. Figure 42 is a late anaphase of a hybrid sporocyte. Thirteen chromosomes plus the m-chromosome (arrow) are visible at the upper pole.

Second division of meiosis is also regular in the hybrids. Occasional tetrad cells were observed in which one or more chromosomes failed to get included in nuclei, but with no higher frequency than in the parents. Cytokinesis is regular, and secondary walls form around the potential spores.

Up to the formation of tetrads, meiotic events in the hybrids are without irregularities. There is total chromosomal compatibility, including the m-pair. No univalents were observed among the regular chromosomes.

Abnormal development in the hybrids begins with spore maturation and differentiation. Some tetrads are arrested in development and abort immediately, while others develop abnormally thickened walls and remain in that condition. Other tetrads break up, and the spores shrivel and disappear, or they may develop a more or less normal wall covering, but fail to enlarge. These may shrivel and disappear; or they may fail to develop chlorophyll, become reddish, and persist in this condition. About half the capsules reach maturity and contain no greenish spores, and about half contain from 2 to 10 spores (Reese & Lemmon, 1965, found as many as 22 green spores in a capsule).

In summary, the high sterility in the spores of the hybrid, *Astomum ludovicianum* × *Weissia controversa*, is not due to meiotic irregularities but is

caused by failure of spores to develop and mature properly following tetrad formation. This would suggest genetic imbalance rather than chromosome imbalance.

8. *Astomum muhlenbergianum* ♀ × *Weissia controversa* ♂

This hybrid was discovered by the late Claire Williams, in heavy clay soil, in a field seeded to permanent pasture, near Thedford, Bosanquet Twp., Lambton County, Ontario, in 1966, and described in detail in a paper published that same year (Williams, 1966). She kindly sent some of this material to us for study, but it was too old for meiotic stages. A second batch of material, sent in 1967, arrived in poor condition, and was overrun with bacteria and molds. Finally, in 1968, material in perfect condition arrived, from which we were able to observe meiosis in the hybrid capsules and in both parental species. The material studied was collected from the same pasture described above (*C. Williams 1555*, March 20, 1968).

The parents and the hybrid are shown in dried condition in Figure 2. The female parent, *Astomum muhlenbergianum*, is at the right (Fig. 2C). It has long, sharply tapering leaves, which are less inrolled than in *Weissia controversa*, and its capsule is completely immersed within the perichaetial leaves. The seta length is less than 0.2 mm; the capsule is subglobose, although this is hard to see in the photograph, because it is so well-hidden in the perichaetium; the capsule is indehiscent; there is no peristome; the exothecial cells are thin-walled; and the spores average 26 μ.

The male parent, *Weissia controversa*, has shorter, mucronate leaves; the capsule is exserted on a seta, which averages 3.5 mm long. The capsule is cylindric, dehiscent; there is a well-developed peristome; the exothecial cells are thick-walled; and the spores average 20 μ.

The hybrid sporophytes are attached to gametophytes which obviously belong to *Astomum muhlenbergianum* (compare Fig. 2B with 2C). The hybrid capsule (Fig. 2B) is on a short seta, averaging 1.2 mm (the hybrid pictured in Fig. 2B, has a seta somewhat shorter than average). Capsules are subglobose and immersed to emergent. Exothecial cells are slightly to moderately thickened. A well-differentiated operculum is present, but it is nonfunctional: it does not dehisce. There is a rudimentary peristome of 16 teeth, each containing one to four articulations. Spores average 22 μ.

The meiotic chromosomes of the two parents are shown in Figures 43 (*Astomum muhlenbergianum*) and 44 (*Weissia controversa*), and each has the number, $n = 13$. The m-chromosome is lacking in both. Meiotic cells of the hybrid are shown in Figures 45 to 47. As in the previous hybrid, meiosis is regular, there are no univalents, and there is an even distribution of chromosomes to the four tetrad nuclei. Figure 47 shows a sporocyte of the hybrid, in anaphase I. Thirteen chromosomes can be seen clearly in both anaphase groups. In prometaphase figures (Figs. 45 & 46), the 13 bivalents are well separated and are all in tight bivalent configurations.

Williams (1966) was unsuccessful in attempts to germinate spores of the hybrid. She obtained no germinations. Only a few spores are produced in

each hybrid capsule, and they are mostly small and reddish. As in the previous hybrid, spore infertility is not due to meiotic irregularities but to failure of the spores to mature and develop normally, following tetrad formation. Again, genetic imbalance is suggested.

DISCUSSION

1. The Nature and Significance of m-chromosomes

It is now well established in many species of mosses that there is polymorphism with respect to the presence or absence of m-chromosomes in different populations. This is contrary to earlier indications and predictions by a number of workers, who based their generalizations upon chromosome counts from a few populations, sometimes on only a single population. In a summary of m-chromosome studies up to that time, Anderson (1964) concluded that diminutive chromosomes (including m-chromosomes) in mosses are not variable in number from individual to individual, but are of constant occurrence with the species. This generalization is completely wrong, as investigations have since shown (Inoue, 1968, 1969; Visotskaya, 1967, 1970; Smith & Newton, 1966, 1967, 1968; Lazarenko & Visotskaya, 1965). The list of species of mosses which have been demonstrated to have populations with different chromosome numbers is long and impressive.

In the three species of *Astomum* and *Weissia* with which we worked, all the plants in an individual population either uniformly contained m-chromosomes or uniformly lacked them, although low frequencies of m-chromosomes in a population might be difficult to detect. The uniformity of chromosome number within a population, even when populations containing the supernumerary were nearby, suggests a very low percentage of outcrossings. It also suggests that clumps of mosses are either clonal or they arise from spores from a single capsule. That these mosses are highly inbred is indicated by these data.

The present studies are the first to indicate that m-chromosome populations are not randomly distributed. Approximately 23% of the populations of *Weissia controversa* in the Atlantic Coastal Plain, of the U.S., contain m-chromosomes; about 12% of the populations in the Piedmont Plateau, and only 2% of populations in the Mountains contain m-chromosomes. These differences are highly significant, and indicate that the presence of m-chromosomes in a higher frequency in the Coastal Plain has selective significance or that m-chromosomes are originating spontaneously and that their rate of origin is higher in the Coastal Plain. Their mode of origin is unknown. We have made yearly checks on a number of populations with and without m-chromosomes in North Carolina and Florida, for a period of four years, and we have found no year to year changes in the chromosomes. We have no evidence that they are accumulating, nor do we have any evidence that they are being eliminated. We can only conclude that they are stable within a population from year to year.

Although we lack quantitative data, there is no question in our minds, after several years of field work, that *Weissia controversa* is more abundant on the

Coastal Plain than in the Piedmont. It is least abundant in the Mountains. Thus, m-chromosome populations are more abundant in the physiographic province in which the species is most abundant. The climatic conditions which seem to favor the moss are mild winter temperatures, high summer temperatures, and possibly open, exposed habitats. Favorable edaphic conditions include sandy, well-drained soils. Sites which favor the most abundant colonization of the moss are sandy soils near the sea coast or sandy banks in the Sand Hills regions of the Coastal Plain. Piedmont soils are composed mainly of heavy, compact clays. Sandy, alluvial soils in the Piedmont are mainly confined to bottomlands and flood plains, where there is too much shade to support *W. controversa* in quantity. In the Mountains, *W. controversa* is uncommon to rare, and the habitats are more likely to be rock crevices, or thin soil over rocks. Temperatures are lower, rainfall is higher, and in general, the sites are more mesic.

A remarkable parallel to m-chromosome distribution in *Weissia controversa* is found in the grasshopper, *Myrmeleotettix maculatus* (Thunb.), in Great Britain, which was investigated by John and Hewitt (1965*a*, *b*) and by Hewitt and John (1967). They found that the number of populations of the grasshopper which contained B-chromosomes decreased from southern to northern Britain, although some populations in the south also lack B-chromosomes. In other words B-chromosomes were found preferentially in populations of *M. maculatus* occupying warm, dry habitats. They were present in reduced frequency or absent altogther in habitats which are either colder or wetter or both. This is precisely the situation in m-chromosome distributions in *W. controversa* in southern and southeastern United States, although our data lack the elegant statistical sophistication provided by the British authors.

Hewitt and John (1967) found that pronounced increase in chiasma frequency accompanied the presence of B-chromosomes in *Myrmeleotettix maculatus*. They argued, therefore, that the value of these supernumerary chromosomes "lies in their capacity to produce increased variability through the development of novel and experimental genotypes under optimal or near optimal environments." These workers are careful to point out, however, that B-chromosomes add to the range of variability of the population rather than providing an improvement in the form of immediate variation of the individual. We are unable to determine chiasma frequencies in *Weissia controversa*, so it is not known whether m-chromosomes influence chiasma frequencies. Any device, however, that would increase genetic variability, even slightly, in a moss like *W. controversa*, which appears to have such low outbreeding capabilities and that propagates vegetatively with such ease, should be selective. We need to know more about population dynamics in mosses and especially the mode of spread of populations, the degree of spread by spores *vs.* vegetative propagation, and some of the elements in short and long range dispersal.

As the m-chromosomes of more and more species of mosses are found to be supernumerary, the question arises, are they different from B-chromosomes? Inoue (1968) has recently adopted the term B-chromosome for m-chromosome in mosses (or for h-chromosomes, as the Japanese have rather uniformly

labelled them), without any discussion of reasons for or against. A large number of species of mosses remains in which m-chromosomes have been observed in all the populations that have been studied. Mehra and Khanna (1961) attempted to distinguish between m-chromosomes and accessory chromosomes, a term introduced by Håkansson (1945) and Müntzing (1945), to more or less substitute for the term B-chromosome. Mehra and Khanna would restrict the term m-chromosome to those species in which it is a regular member of the complement, that is, when it is not a supernumerary. This distinction was also suggested by Lewis (1961), who realized there may be two types of diminutive chromosomes in mosses. Until more thorough studies have been made of these small chromosomes, however, it seems best to retain the term m-chromosome until more precise characterizations can be found.

Not enough populations of either species of *Astomum* were studied to determine whether populations with m-chromosomes are distributed randomly or non-randomly. Similarly, it was not possible to determine whether all of the individuals in each population have the same chromosome number. As already noted, relatively large numbers of individuals must be sampled from a population to detect a low frequency of plants with the supernumerary. If plants with m-chromosomes comprise 10% of the population, it requires a sample of 45 to detect m-chromosomes in the populations with even a 5% significance level. Since we studied only ten collections of *A. muhlenbergianum*, it is remarkable that three different chromsome numbers were discovered.

2. Polyploidy

Polyploid populations were found in all three species investigated. The polyploid populations of *Weissia controversa*, as we have already stated, have morphological distinctions and even habit characteristics that we think may merit specific rank. Furthermore, all of these polyploids were restricted to a unique habitat, namely limestone and dolomitic cedar (*Juniperus virginiana*) outcrops in the Blue Ridge, Valley, Cumberland, and Ozarkian Provinces. We are currently studying these populations.

Of the 460 populations of typical *Weissia controversa*, not a single tetraploid was found. From this, we conclude that ploidy is not recurring frequently, or if so, the plants are not becoming established.

The tetraploids in *Astomum ludovicianum* and *A. muhlenbergianum* are indistinguishable from the diploids. Tetraploids in both species possess the same range of leaf cell sizes and spore sizes, and no differences could be found in their relative vigor. Löve claims that "types with different euploid chromosome numbers can always be separated by an unbiased student." As Lewis and John (1963) point out, however, this may be true for experimental material, but there are many exceptions among natural polyploids, even allopolyploids. Stebbins (1950) lists several examples of extreme allopolyploids which may resemble one or the other of their parental species so closely that they have not been recognized as distinct by systematists. Dozens of species of mosses can be cited in which there are indistinguishable tetraploid and diploid populations. Among these are *Distichium capillaceum*, $n = 14$ and 28

(Visotskaya, 1967; Anderson & Crum, 1958); *Ditrichum pallidum*, n = 13, 26 (Al-Aish & Anderson, 1961); *Dicranum fuscescens*, n = 12, 24 (Anderson & Crum, 1958); *Octoblepharum albidum*, n = 13, 26 (Khanna, 1960*c*); *Hypoptergium rotulatum*, n = 9, 18, 27, 36 (Ramsay, 1967), *etc.* (see Anderson, 1964).

Autopolyploids in higher plants tend to be highly sterile, usually because of multivalent associations of chromosomes in meiosis, although many exceptions to this have been listed by Stebbins (1950), Lewis and John (1963), and others. Unfortunately, no one has carried out germination tests of tetraploid populations in mosses, but thus far, all of the natural tetraploid populations studied have been found to undergo normal meiosis. Bivalent formation is the rule, and, at most, secondary associations of bivalents have been described. Even in an experimentally produced autotetraploid, Wettstein and Straub (1942) reported normal bivalent formation. This is also the case in both *Astomum muhlenbergianum* and *A. ludovicianum*. It seems probable that somehow mosses have adjusted their meiotic mechanism in such a way that the duplicated bivalents do not form multivalents. Lewis and John (1963) call attention to the fact that larger chromosomes tend to form multivalents more than smaller ones and that multivalents are formed more often where there is a high frequency of distributed chiasmata. No data are available on chiasma frequency in mosses. It is probably low, judging from bivalent configurations observed at prometaphase and metaphase I. Also, the amount of heterochromatin in moss chromosomes appears to be high, compared to many plants, and there may be more achiasmic bivalents than is suspected. The smallness of moss chromosomes has been repeatedly mentioned. In addition to these physical factors, however, there are probably intrinsic genetic influences that operate against multivalent formation.

3. Hybridization and Sterility Barriers

Several natural intergeneric hybrids have been described in mosses that parallel features of the *Astomum* × *Weissia* hybrids. The most notable, perhaps, are between cleistocarpous and stegocarpous genera in the Funariaceae, which were first described by Britton (1895) and Andrews (1918), both of whom reported finding natural hybrids between *Physcomitrella patens* × *Physcomitrium turbinatum*. Wettstein (1924) produced comparable hybrids experimentally, by crossing *Physcomitrella patens* × *Physcomitrium euryostomum*, and *Physcomitrella patens* × *Funaria hygrometrica*. The latter two hybirds have also, from time to time, been observed in nature. More recently, Andrews and Hermann (1959), reported natural hybrids between *Pleuridium acuminatum*, also a cleistocarpous moss, and *Ditrichum pallidum*, which has an operculum and a well-developed peristome.

Represented by these intergeneric hybrids are three families of mosses that are relatively unrelated, *Ditrichaceae*, *Pottiaceae* and *Funariaceae*, although the former two families are more closely related to each other than to the latter. Yet, each of the hybrid combinations shares the following characteristics: (1) one parent is cleistocarpous, the other is stegocarpous; (2) reciprocal

hybrids have not been found in nature; (3) each hybrid capsule produced few or no spores, and the few spores produced are highly sterile; (4) meiosis is regular in the hybrids, pairing of chromosomes is complete, and no univalents are present (*Pleuridium-Ditrichum* hybrid has not been studied cytologically); and (5) despite production of a few fertile spores by each hybrid, no F_2 plants have ever been found in nature, and there is no evidence of introgression among the genera and species involved.

The fact that the chromosome complements among species of closely related genera are similar in number and apparent morphology, and that they pair with ease when brought together in a hybrid combination probably indicates a minimum of major structural changes and rearrangements in the complement during evolution. The imbalance in hybrids is intrinsically genic, rather than chromosomal. The hybrid chromsomes pair and segregate with no difficulty, but the new genic combinations brought together in the respective cells of the tetrads create an imbalance that leads to sterility in nearly all of them. Even the so-called fertile spores which are produced and are able to germinate apparently do not grow into mature plants. At least they are never found in nature. Thus, in spite of the ease with which F_1 hybrid sporophytes are produced, the sterility barrier appears to be total.

Wettstein (1924), who found complete chromosome compatibility in hybrid sporophytes, *Physcomitrella patens* × *Physcomitrium turbinatum*, and nearly complete sterility, found occasional tetrads in which two of the spores were viable, and the other two were sterile. He was able to grow the two viable spores to maturity, but found that the two plants they produced were identical with the maternal parent. Wettstein hypothesized that the two viable spores contained a complete set of maternal chromosomes, which had been segregated at meiosis as a complete maternal genome. The two spores which received the paternal set were sterile, according to Wettstein, because the paternal set of chromsomes can not function in maternal cytoplasm.

If Wettstein's hypothesis is correct, namely, that the few viable spores which are produced by these hybrids contain a complete set of maternal chromosomes, then this would explain the lack of F_2 plants in natural populations. Viable spores from the hybrids would always grow into plants indistinguishable from the maternal species. However, this hypothesis requires an explanation to account for the preferential segregation of complete sets of parental genomes. There are 27 pairs of chromosomes in the *Physcomitrella-Physcomitrium* hybrids, and 13 pairs in the *Astomum-Weissia* and *Pleuridium-Ditrichum* hybrids. The probability of segregation of complete parental sets by chance is too low to account for even a few tetrads per capsule. Some mechanism must be postulated to provide a slight preference for segregation of whole sets.

Another explanation that has been advanced to explain sterility in hybrids which have normal meioses is that sterility is caused by structural hybridity involving small chromosomal segments. Originally suggested by Sax (1933), Stebbins (1950) has proposed the term cryptic structural hybridity for this type of sterility, and Stebbins lists a number of higher plants in which this explanation might apply. Sterility is presumably caused by heterozygosity

for structural differences so small as not to influence materially chromosome pairing. The origin of such tiny structural differences involves a series of inverted chromsome segments followed by re-inversions in which the breaks are almost but not quite at the same places on the chromosome (Müntzing, 1930; Sturtevant, 1938).

Stebbins (1958) has stressed the difficulties involved in distinguishing between genic and chromosomal sterility, especially when the latter involves such small structural differences that chromosomes pair normally at meiosis. The only reliable criterion for distinguishing between them is that furnished by chromosome doubling. If an *Astomum-Weissia* hybrid can be found with the number, $n = 26$, and if its sterility is significantly reduced in comparison with the diploid hybrids, then sterility in the latter is due to chromosomal imbalance. If, on the other hand, doubling does not produce increased fertility, genic imbalance is indicated.

4. Isolating Mechanisms and Generic Limits

There is a set of remarkable parallelisms surrounding the *Astomum-Weissia*, *Pleuridium-Ditrichum*, *Physcomitrium-Physcomitrella-Funaria* hybrids that have been referred to above. These parallels are discussed below.

First, within each complex, the gametophytes of the hybridizing species are closely alike, morphologically. In each cross, one parent is cleistocarpous, the other parent is stegocarpous, and is either peristomate or gymnostomous. In the evolution of mosses, capsule reduction has taken place independently in a number of diverse families. The reduction sequence is presumed to be: operculate and peristomate → operculate and gymnostomous → operculate, but non-functional → cleistocarpous. In the three complexes, the sequence is represented by the following genera: *Weissia* → *Hymenostomum* → *Astomum*; *Ditrichum* → *Pringleella*, et al. → *Pleuridium*; *Funaria* → *Physcomitrium* → *Aphanorrhegma* → *Physcomitrella*.

Second, the regular chromosome complement of the species of each complex is identical, except in the *Funaria* complex. Most species of *Funaria* have the number $n = 28$ (14), instead of $n = 27$, as in *Physcomitrium* and *Physcomitrella*. There is complete pairing of chromosome sets in each of the hybrids which has been studied (*Pleuridium-Ditrichum* hybrids are unstudied), and meiosis in each hybrid is apparently regular.

Third, although meiosis in the hybrid capsules is regular and produces normal-appearing tetrads, in nearly all of the latter, spore development is arrested or the spores abort, and the capsules produce few or no spores.

Fourth, although the species within each of the three complexes hybridize with seeming ease, neither F_2 nor backcrosses have been observed in nature. Nearly all of the parental species involved are notoriously variable, morphologically, but they are taxonomically distinct, and intergradations in the field are unknown. They are not "problem" species, except, perhaps, at subspecific levels. The lack of apparent introgression indicates the effectiveness of the sterility barriers.

These parallels suggest that the same isolating mechanisms may be operating in each of the complexes. Whether genic or chromosomal imbalances or a combination of the two are responsible for the high degree of haplontic sterility cannot be determined at this point. The role of spatial isolation in these organisms is almost entirely unknown. The caespitose or clump habit of *Astomum* and *Weissia*, and doubtless other genera, may provide a major isolating device by forcing interbreeding among plants within the clump. Until the origin or origins of clumps are known, the degree of spatial isolation it provides cannot be estimated. Local and wide-range dispersal patterns and the incidences of asexual *vs.* sexual reproduction are in need of study.

The fact that intergeneric hybridization occurs between species of *Astomum* and *Weissia*, and because the gametophytes of species in the two genera are almost indistinguishable, Reese and Lemmon (1965) merged *Astomum* with *Weissia*, taxonomically, and made the necessary nomenclatural transfers. Claire Williams (1966), after finding intergeneric hybrids between species of the same genera, arrived at the same conclusion.

The close similarities of chromosome complements and the regular pairing of chromosomes in hybrid meioses indicate that *Weissia controversa*, *Astomum ludovicianum*, and *A. muhlenbergianum* are closely related species. The close relationship between the species which have been placed in the two genera is also borne out by the resemblances of the gametophytes. It should be kept in mind, however, that gametophytic resemblances, in somewhat lesser degree, extend to *Trichostomum*, *Tortella*, and to an even lesser extent to *Timmiella*. A putative hybrid between *Tortella flavovirens* and *Astomum crispum*, described by Nicholson (1910), should be recalled.

Despite the similarities in chromosome complements and in morphological characteristics of the gametophytes, however, the isolation barriers between the two species of *Astomum* and *Weissia controversa* are highly effective. Equally effective barriers may exist between *A. ludovicianum* and *A. muhlenbergianum*, although we have no information. Hybrids between the latter have not been reported.

Cleistocarpy, as a taxonomic character, should carry considerable weight, especially since it represents a rather significant evolutionary step in capsule reduction. Also, its development involved more than a few trivial genic alterations, since it has been accompanied by a high degree of genetic isolation. Stebbins (1950) cites many examples in which two species may differ in a very large number of gene differences or even by as many as 30 to 50 translocated or inverted chromosomal segments, yet, when hybridized, the offspring have regular pairing and a high degree of haplontic sterility.

The final judgment, therefore, as to whether *Astomum* is worthy of generic rank, must be made primarily upon morphological assessments. Detailed morphological analyses of all of the described taxa in the complex should be made. A competent evaluation of generic groupings can then be made in the light of cyto-genetical information which we have presented in this paper. In the meantime, attempts at experimental hybridization and analyses of population dynamics in mosses are long overdue.

Summary

1. Cytogeographical studies were carried out on 460 populations of *Weissia controversa*, 17 populations of *Astomum ludovicianum*, and 10 populations of *A. muhlenbergianum*. Polymorphism as to chromosome number was found in all three species: *W. controversa*, n = 13 and n = 13 + m; *A. ludovicianum* and *A. muhlenbergianum*, n = 13, n = 13 + m, and n = 26.

2. Populations of *Weissia controversa* containing m-chromosomes are non-randomly distributed. In the Atlantic Coastal Plain, the m-chromosome frequency is approximately 23%. In the Piedmont Plateau, the m-frequency is 12%, and in the Mountains it is less than 3%. The over-all frequency of m-chromosome populations is 16%.

3. Morphological analyses were carried out on selected plants from populations with each of the chromosome numbers. No correlations exist between chromosome number and morphology. Any effects that m-chromosomes exert must be endophenotypic.

4. The varying configurations that m-chromosomes display during prometaphase and metaphase I of meiosis can be explained by assuming they are achiasmic, and they thus reveal various degrees of pairing and desynapsis, including configurations of bivalents, univalents, half-bivalents, chromatids, and their derivative combinations.

5. Meiosis was studied in two intergeneric hybrids, *Astomum ludovicianum* ♀ × *Weissia controversa* ♂, and *A. muhlenbergianum* ♀ × *W. controversa* ♂. In both hybrids meiosis was regular and there was complete pairing of chromosomes. No univalents or other irregularities sometimes associated with hybrids were observed.

6. Hybrid sporophytes in both crosses produced few spores. Fertility among the few spores produced is very low, from 0 to 2%, and no leafy plants were produced, although failure of the latter may have been due to poor cultural conditions.

7. Normal-appearing tetrads are produced by the hybrids, but spores fail to develop or they develop partially and abort. Sterility results from either chromosome imbalances brought about by very small structural differences that do not impair synapses or by genic imbalances in the segregated haploid sets. It was not possible to determine which of these imbalances produces the haplontic sterility.

8. The close resemblances of the standard chromosome complements and their close compatibility in hybrid meioses suggest a close relationship among the three species, but the high degree of sterility of the hybrids and the absence of introgression suggest highly effective isolation barriers. It is suggested therefore, that a taxonomic evaluation of *Astomum* await a world-wide systematic revision of the entire complex.

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